Role of electrostatic interactions in protein – protein complex formation: direct electrochemistry of complexes involving tetraheme cytochrome c3

É. Lojou, M. Guiral-Brugna, M.-T. Giudici-Orticoni, M. Bruschi, P. Bianco
BIP-CNRS
31 chemin Joseph Aiguier
13402 Marseille Cedex 20 France

Electron transfer reactions between redox proteins are believed to involve the formation of precursor complexes which are stabilized and oriented by electrostatic and hydrophobic interactions. Amino acids of opposite charges on each partner protein participate to the formation of electrostatic bonds. Among redox proteins complexes, those between cytochrome c and cytochrome b5 or plastocyanin have been extensively studied for a long time [1,2]. More recently, several papers described the characterization and studied the stability of complexes between physiological redox partners (e.g. cytochrome c3 and hydrogenase) extracted from sulfate reducing bacteria [3-5]. However, the extend of electrostatic effects in protein – protein recognition has not yet been clearly addressed. Although hydrophobic interactions are supposed to be necessary for the electron transfer process between the prosthetic groups, it has been suggested that the electrostatic interactions are the driving force in the specificity of metalloprotein complex formation.

C-type cytochromes have been well investigated in order to try to unravel the factors that govern the rate and the specificity of protein/protein electron transfer. Cytochromes c3 are tetraheme proteins acting as fast electrochemical systems at various working electrodes, and exhibiting very low redox potentials. They are present in all the anaerobic sulfate reducing bacteria of *Desulfovibrio* genus. An interesting feature is the existence of several cytochrome c3 extracted from these bacteria varying in isoelectric point i.e. in global electric charge. Among them, cytochrome c3 from *Desulfovibrio vulgaris* Hildenborough (DvH) is a privileged candidate for investigating electrostatic effects because of its excess of positive charges at physiological pH (pI of about 10.5).

In this communication, we describe results on the electrochemical study of the interaction between DvH cytochrome c3 and other redox proteins present in electron-carrier chains as physiological (e.g. hyrogenases or hexadecaheme high molecular weight cytochrome c abbreviated as Hmc) or not physiological partners. It is demonstrated that DvH cytochrome c3 can promote the electrochemical reduction/re-oxidation of several proteins (flavodoxin, ferredoxin, “acid” cytochrome c3 from *Desulfovibrio africanus*, etc.). An essential feature is that the heme crevice of cytochrome c3 which is supposed to interact with redox partners is surrounded by basic (lysine) residues. In each case, it is suggested that a complex is formed which is favorably orientated for electron exchanges to be achieved. Complex formation is well characterized by other additional approaches such as cross-linking and kinetics analysis experiments in the case of cytochrome c3 and Hmc.

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